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Molecular techniques based on ITS analysis: useful tools for the identification of wood-decay Basidiomycetes in urban trees?

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Summary

C. A. Robles, C. C. Carmarán & S. E. Lopez. 2012. Molecular techniques based on ITS analysis: useful tools for the identification of wood-decay Basidiomycetes in urban trees? *Kurtziana* 37 (1): 91-108.

The presence and identity of wood-decay Basidiomycetes affecting *Platanus acerifolia* were evaluated in Buenos Aires City during 2007. From 247 samples of basidiomes and wood with signs of decay, 19 strains were isolated, from which 10 rDNA sequences were obtained. The aim of this work was to analyze the scope of information obtained from ITS sequences as taxonomic tools to study local populations of wood-rotting fungi in urban trees. Phylogenetic analyses were performed under static and dynamic homologies. The techniques employed enable to: identificate *Coprinellus micaceus* and corroborate the identification of *Bjerkandera adusta*, while these same results were not achieved for *Inonotus rickii*, *Ganoderma resinaceum*, *Peniophora laxitexta* and *Phlebiopsis gigantea* due to the complexity of the genera and the lack of sequences. Lack of sequences at a regional level would be a major obstacle for the detection of wood decay agents in urban trees using ITS sequences.

Key words: Wood-decay fungi; Urban trees; Phylogenetic analyses; *Bjerkandera adusta*; *Coprinellus micaceus*; *Ganoderma resinaceum*; *Inonotus rickii*; *Peniophora laxitexta*; *Phlebiopsis gigantea*.

Resumen

C. A. Robles, C. C. Carmarán & S. E. Lopez. 2012. Técnicas moleculares basadas en análisis de ITS: ¿herramientas útiles para la identificación de Basidiomycetes degradadores de la madera en árboles urbanos? *Kurtziana* 37 (1): 91-108.

Durante 2007 se evaluó la presencia e identidad de Basidiomycetes xilófagos que afectan a *Platanus acerifolia* en la Ciudad de Buenos Aires. Sobre la base de 247 muestras de basidiomas y madera con signos de pudrición se aislaron 19 cepas, a partir de las cuales se obtuvieron 10 secuencias correspondientes a rADN. El objetivo de este trabajo fue analizar la utilidad de la información brindada por las secuencias correspondientes al fragmento ITS en la identificación taxonómica de poblaciones locales de hongos xilófagos en árboles urbanos. Se realizaron análisis filogenéticos bajo homologías estáticas y dinámicas. Los resultados obtenidos fueron comparados con las identificaciones morfológicas y de cultivo previas de las cepas. Las técnicas empleadas permitieron: identificar a *Coprinellus micaceus* y corroborar la identificación de *Bjerkandera adusta*, mientras que no se lograron los mismos resultados, debido a la complejidad de los géneros y a la ausencia de secuencias, para *Inonotus rickii*, *Ganoderma resinaceum*, *Peniophora laxitexta* y *Phlebiopsis gigantea*. La falta de secuencias a nivel regional aparece como uno de los principales obstáculos para la detección de agentes de pudrición de la madera en árboles urbanos, mediante los análisis basados en secuencias ITS.

Palabras clave: Hongos de pudrición; Árboles urbanos; Análisis filogenéticos; *Bjerkandera adusta*; *Coprinellus micaceus*; *Ganoderma resinaceum*; *Inonotus rickii*; *Peniophora laxitexta*; *Phlebiopsis gigantea*.

Introduction

Urban trees provide invaluable benefits for well-being of people, and their phytosanitary condition is an important issue in the urban planning. Understanding the fungi involved in tree damage can be useful to estimate the damage severity. Detection and identification of decay fungi by molecular tools were used in several recent studies (Adair et al., 2002; Jellison et al., 2003; Nicolotti et al., 2009). They are a promising alternative for specific, sensitive and rapid routine diagnoses. PCR-based methods using nuclear or mitochondrial ribosomal DNA (rDNA) loci have proven valuable for fungal detection and identification at different taxonomic levels (Guglielmo et al., 2007; 2010). One of the present trends concerning diagnosis of wood rot is the analysis of fungal DNA extracted directly from wood (Hoegger & Kües, 2007). At the same time, few studies involving DNA techniques were carried out in relation with fungi in urban trees (Guglielmo et al., 2007; 2008; 2010; Robles et al., 2011).

London plane (*Platanus acerifolia*) is one of the most frequent urban tree species in Buenos Aires City (Filippini et al., 2000) and it is one of the most affected species by fungal pathogens (Sede & Lopez, 1999).

The aim of this work was to analyze the scope of information obtained from ITS sequences as taxonomic tools to study local populations of wood-rotting fungi in urban trees.

Material and methods

A survey was carried out in Buenos Aires City during 2007. Wood samples, basidiomes and conidiomata were removed from a total of 247 London plane trees showing cankers, cavities or debarking areas in their trunks or lower branches (Robles et al., 2011). All the samples obtained were processed and plated onto Malt Extract Agar (MEA) 1.2%. The cultures were examined every 2-3 days in order to detect Basidiomycetes isolations. The criteria applied for the selection of potential Basidiomycetes strains were the lack of sporulation, the presence of clamp connections and the appearance of the colony. A total of 19 axenic strains were obtained and identified by means of keys based on mycelial characters (Deschamps & Wright, 1975; Nobles, 1965; Stalpers, 1978) and morphological characters of basidiomes. These strains were incorporated to the culture collection of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (BAFCcult).

A total of sixteen strains were selected for DNA extractions and the ITS1 and ITS2 regions were amplified. The nucleotide sequences determined in the present study were deposited in the GenBank DNA sequence database. Their accession numbers are listed in Tables 1-6.

For genotypic analyses, the cultures used for the molecular studies were grown on MEA 1.2%. The cultures were incubated in the dark at 25°C for 21 days. DNA was extracted from the cells by using the UltraCleanTM Microbial DNA Isolation Kit (MO BIO laboratories inc., Solana Beach, USA), according to the manufacturer's instructions. The ITS region of the strains was amplified by using the universal primers ITS1 and ITS4 (White et al., 1990) under the following PCR conditions: (1) 94 °C for 5:00 min; (2) 94 °C for 1:00 min; (3) annealing at 58 °C for 1:00 min; (4) extension at 72 °C for 1:30 min; (5) 35 times to 2; and (6) a final extension of 72 °C for 7:00 min. PCR reaction mix was prepared as follows: dNTPs (1.85 µl) at 1 mM, primers ITS1 and ITS4 (0.5 µl) at 10 mM each, *Taq* polymerase (0.2 µl) at 5 U/µl, $MgCl_2$ (1.25 µl) at 50 mM and the reaction buffer supplied by the manufacturer (2.5 µl). In some cases, best amplification results were achieved by adding 1 µl of 2 % bovine serum albumin (BSA, Promega Corp.) to the PCR reaction mix. PCR products were purified using a QIAGEN Gel Extraction kit (QIAGEN Inc.). Both strands of each fragment were sequenced by Macrogen DNA sequencing service. Sequences obtained were compared with sequences from GenBank (Tables 1-6).

Following the guidelines expressed by Hibbet et al. (2011) and Nilsson et al. (2011), environmental samples of NCBI BLAST were tested in order to identify sequences with high similarity, without significant results.

DNA sequences were edited with the software program BioEdit sequence alignment editor, version 7.0.5.3 (Hall, 1999). A sequence of *Amanita muscaria* (EU346871) was chosen as an outgroup in all the analyses. The phylogenetic analysis of the sequence data under static homologies was performed using the parsimony method and NONA version 2.0 (Goloboff, 1997) with all the characters equally weighted and gaps scored as missing data. To determine the support for each clade, bootstrap analyses were performed with 1000 replications. Phylogenetic analysis under dynamic homologies was performed using the software program POY version 4.0.2467 Beta

(Wheeler, 2006). All the characters were equally weighted and auto_sequence_partition, build (10) and swap (100) were used as parameters. Bremer support values were calculated for each clade.

Results and Discussion

Ten DNA sequences were obtained, which under phylogenetic analyses produced the following results described and discussed below. Some notes about the species recognized are given.

Bjerkandera adusta (Willd.) Karst. Meddn Soc. Fauna Flora fenn. 5: 38, 1879

Culture descriptions: See Nobles (1965) and Stalpers (1978).

Examined material:

ARGENTINA. Buenos Aires City, C. A. Robles, VII-2007. On *Platanus acerifolia*. BAFC cult3301.

The strain BAFCcult 3301 (GenBank nº FJ850965) was identified as *Bjerkandera adusta* by cultural characters. The phylogenetic analyses included sequences from Belice, Canada, China, Japan, Latvia, Lithuania, Poland and Sweden.

Analysis under static homologies resulted in three most parsimonious trees, tree length = 416, CI = 0.94, RI = 0.97. Analysis under dynamic homologies resulted in one retained tree, Cost = 749 (Fig. 1).The results suggest a division of the strains in two main clades without geographical or host correlation. The Argentinean strain appears in the same clade that a Sweden one from *Picea abies* (Vasiliauskas et al., 2005) with low support.

This is first sequence reported from Argentinean collection of *B. adusta*.

The position of the strain assigned to Bjerkandera fumosa (GenBank nº FJ903396) suggests a misidentification.

Coprinellus micaceus (Bull.) Vilgalys, Hopple & Jacq. Johnson, *Col. Fig. Engl. Fung.* Vol. 3, pl. 261, 1800

Culture description: see Robles et al. (2011)

Examined material:

ARGENTINA. Buenos Aires City, C. A. Robles, I, V, VIII-2007. On *Platanus acerifolia*. BAFC cult 3218 (GenBank nº FJ850970), BAFC cult 3311 (GenBank nº FJ850971), BAFC cult 3312 (GenBank nº FJ850969), BAFC cult 3313 (GenBank nº FJ850968).

Phylogenetic analyses enable identification since it was not possible to obtain basidiomes and there were not previous culture descriptions of this species. We included sequences from Australia, China, Czech Republic, Hawaii, Spain, Sweden and USA. Analysis under static homologies resulted in twenty-eight most parsimonious trees, tree length = 930, CI= 0.61, RI = 0.79. Analysis under dynamic homologies resulted in two retained trees, Cost = 1725. The results suggest division of clades in relation with the species involved without a geographical correlation (Fig. 2). Analyses under both static and dynamic homologies show that all Argentinean strains are located in the same clade, together with members of *C. micaceus*, from Australia, Hawaii, Spain and unknown origins.

Ganoderma resinaceum Boud., in Patouillard, *Bull. Soc. mycol. Fr.* 5: 72, 1890 [1889]

Culture descriptions: See Bazzalo & Wright (1982) and Stalpers (1978).

Examined material:

ARGENTINA. Buenos Aires City, C. A. Robles V, XII-2007. On *Platanus acerifolia*. BAFC cult 3296, BAFC cult3297.

The strains corresponding to BAFCcult 3296 (GenBank nº FJ850967) and 3297 (GenBank nº FJ850966) were identified as *Ganoderma resinaceum* by cultural and morphological characters. The phylogenetic analyses included sequences from China, England, Finland, Germany, Netherlands and USA. Analysis under static homologies resulted in five most parsimonious trees, tree length = 366, CI = 0.7, RI = 0.81. Analysis under dynamic homologies resulted in four retained trees,

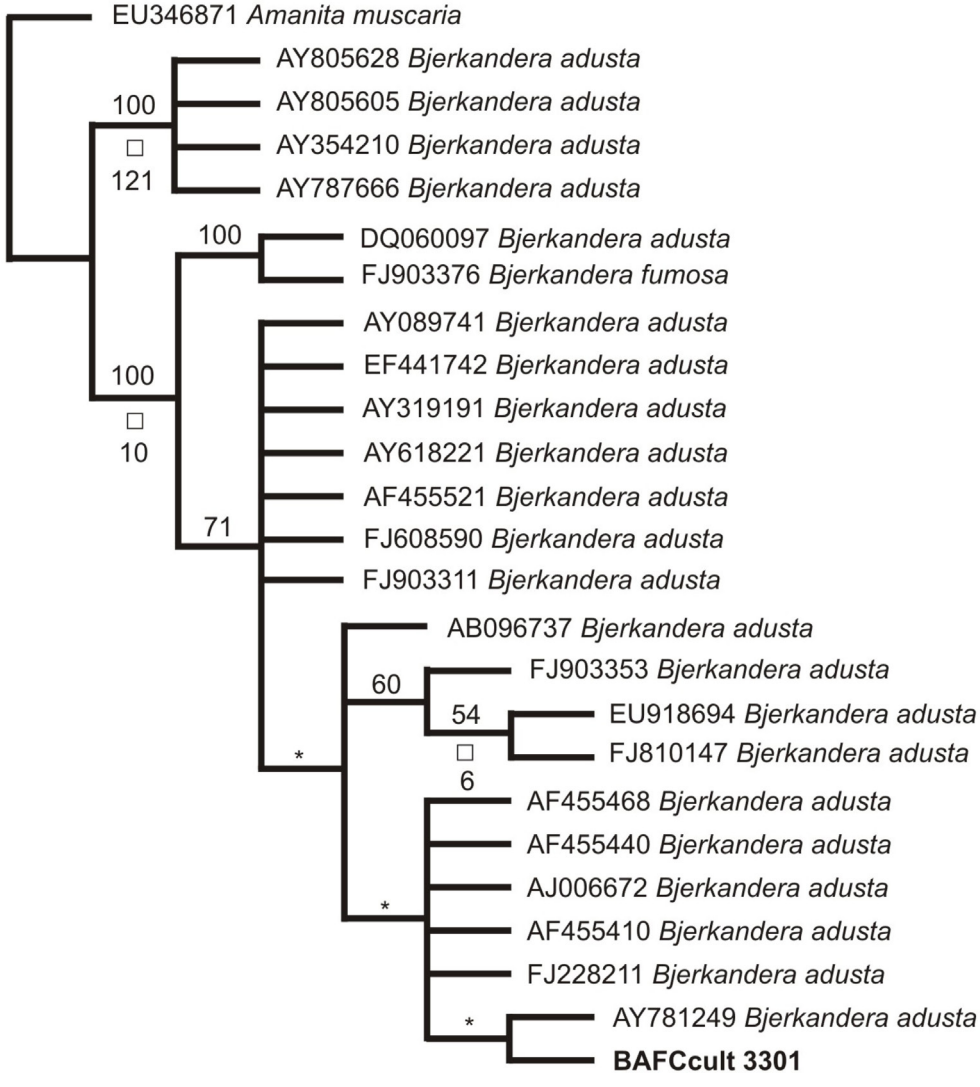

Fig. 1. The strict consensus trees obtained from *Bjerkandera* sequences based on ITS sequence data and maximum parsimony analysis under static homologies. Boostrap support values for 1000 replications are shown above branches. Bremer support values for default values are shown below branches. * indicates collapsed clades when Boostrap support was performed. □ indicates clades recovered by both analyses under static and dynamic homologies. In bold type is presented the sequence obtained in this work. The tree was rooted to *Amanita muscaria* (EU346871).

Cost = 1048. The results suggest a division of clades without a clear relation with the species involved. Analyses show a clade formed with strains of *G. resinaceum* and *Ganoderma pfeifferi*; a second clade containing strains of *Ganoderma lucidum* and *Ganoderma tsugae* and a third clade formed with strains of *Ganoderma tsugae*, *Ganoderma australe* and *Ganoderma adspersum*. Argentinean strains locate separately from European and Chinese *G. resinaceum*'s strains, in a separate clade from all other species. There are partial ITS sequences of *Ganoderma* strains from Argentina. However, these were not detected by BLAST and due to their small size they were not considered in the analyses. Taking into consideration the macro and micro-morphological characters and given the complexity of the relations inside the

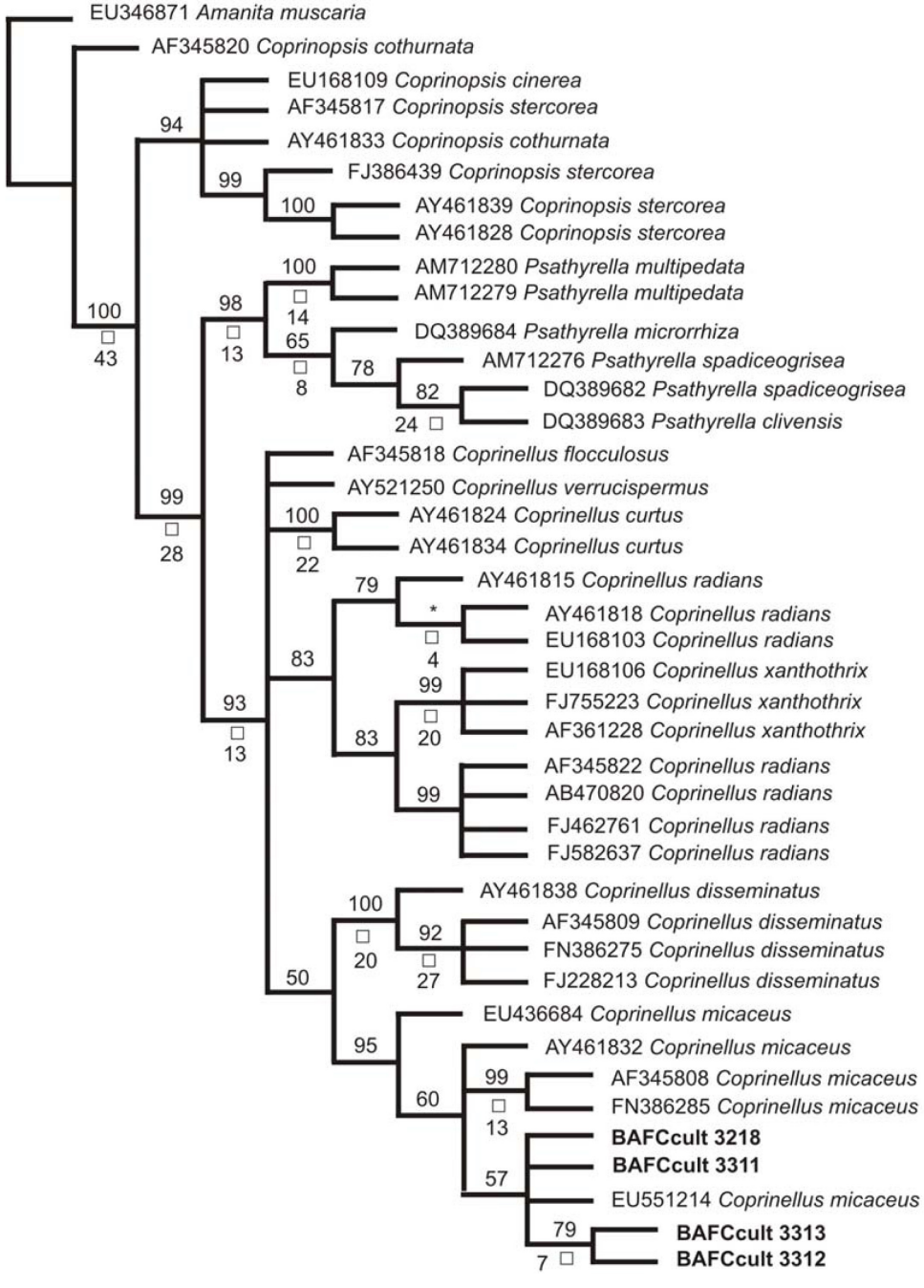

Fig. 2. The strict consensus trees obtained from *Coprinellus*, *Coprinopsis* and *Psathyrella* sequences based on ITS sequence data and maximum parsimony analysis under static homologies. Boostrap support values for 1000 replications are shown above branches. Bremer support values for default values are shown below branches. * indicates collapsed clades when Boostrap support was performed. □ indicates clades recovered by both analyses under static and dynamic homologies. In bold type are presented the sequences obtained in this work. The tree was rooted to *Amanita muscaria* (EU346871).

genus, already noted by Gottlieb et al. (2000), a conservative position was adopted and the strains of this study were located inside the species *Ganoderma resinaceum*. The above-mentioned complexity might be solved by testing the level of resolution of alternative genes (Fig. 3).

Inonotus rickii (Pat.) D.A. Reid, Kew Bull. 12: 141, 1957.

Culture description: See Wright & Iaconis (1955).

Examined material:

ARGENTINA. Buenos Aires City, C. A. Robles, V, IX-XII-2007. On *Platanus acerifolia*. BAFC cult 3319.

This *Inonotus* strain (BAFCcult 3319; GenBank nº GU016326), showed cultural and morphological characters similar to those of *I. rickii* (Robles et al., 2011). The phylogenetic analyses included sequences from Argentina, Canada, China, Germany, Spain and USA. Analysis under static homologies resulted in five most parsimonious

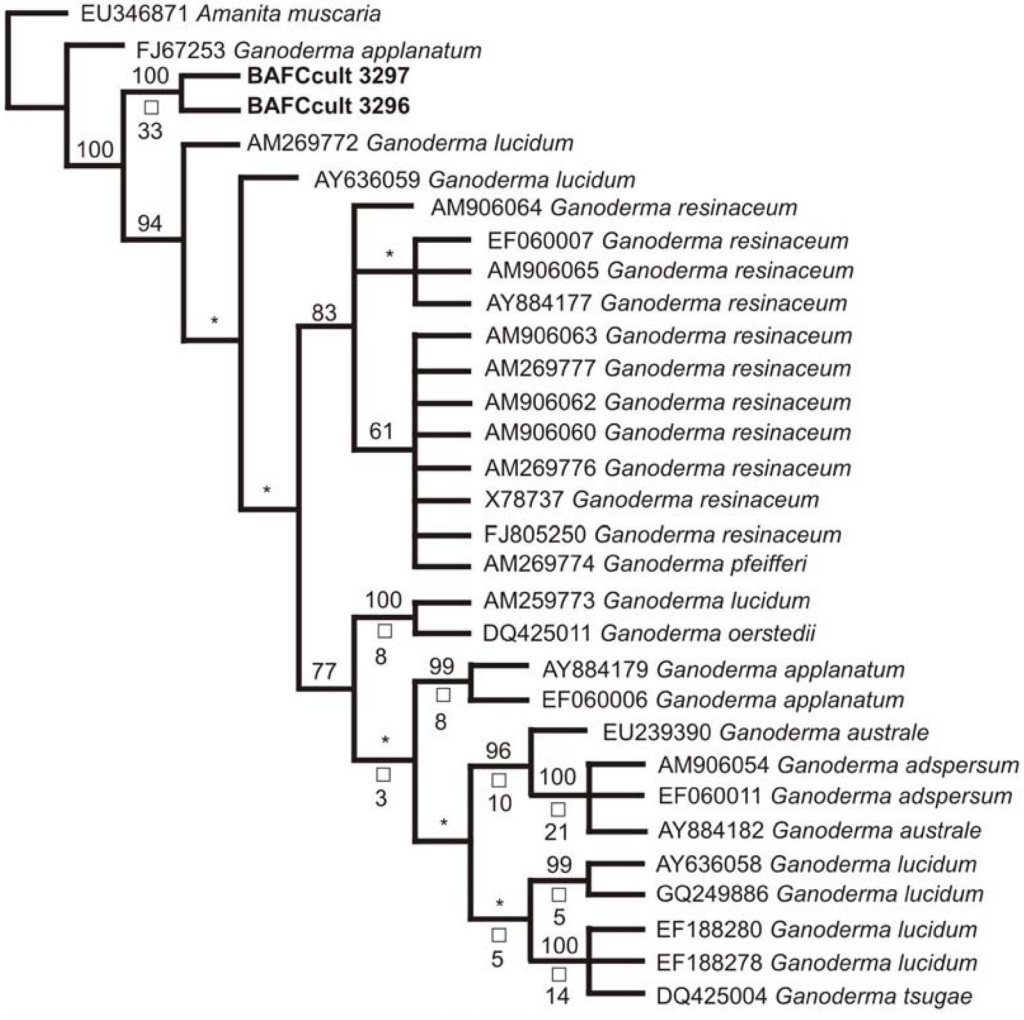

Fig. 3. The strict consensus trees obtained from *Ganoderma* sequences based on ITS sequence data and maximum parsimony analysis under static homologies. Boostrap support values for 1000 replications are shown above branches. Bremer support values for default values are shown below branches. * indicates collapsed clades when Boostrap support was performed. □ indicates clades recovered by both analyses under static and dynamic homologies. In bold type are presented the sequences obtained in this work. The tree was rooted to *Amanita muscaria* (EU346871).

trees, tree length = 1248, CI = 0.57, RI = 0.65. Analysis under dynamic homologies resulted in one retained tree, Cost = 2135. Under static homologies, the strain of this study is located in the same clade as the rest of the strains of *I. rickii*, these from China and Argentina, together with two strains of *Inonotus patouillardii*, one of them from Argentina (Fig. 4). These results support those obtained by Gottlieb et al. (2002) and indicate that this technique is not able to solve the conflict between *I. rickii* and *I. patouillardii*'s identification.

Peniophora laxitexta C.E. Gómez, Darwiniana 20(1-2): 195, 1976.

Culture description: See Gómez & Loewenbaum (1976).

Examined material:

ARGENTINA. Buenos Aires City, C. A. Robles, IX-2007. On *Platanus acerifolia*. BAFC cult 3309.

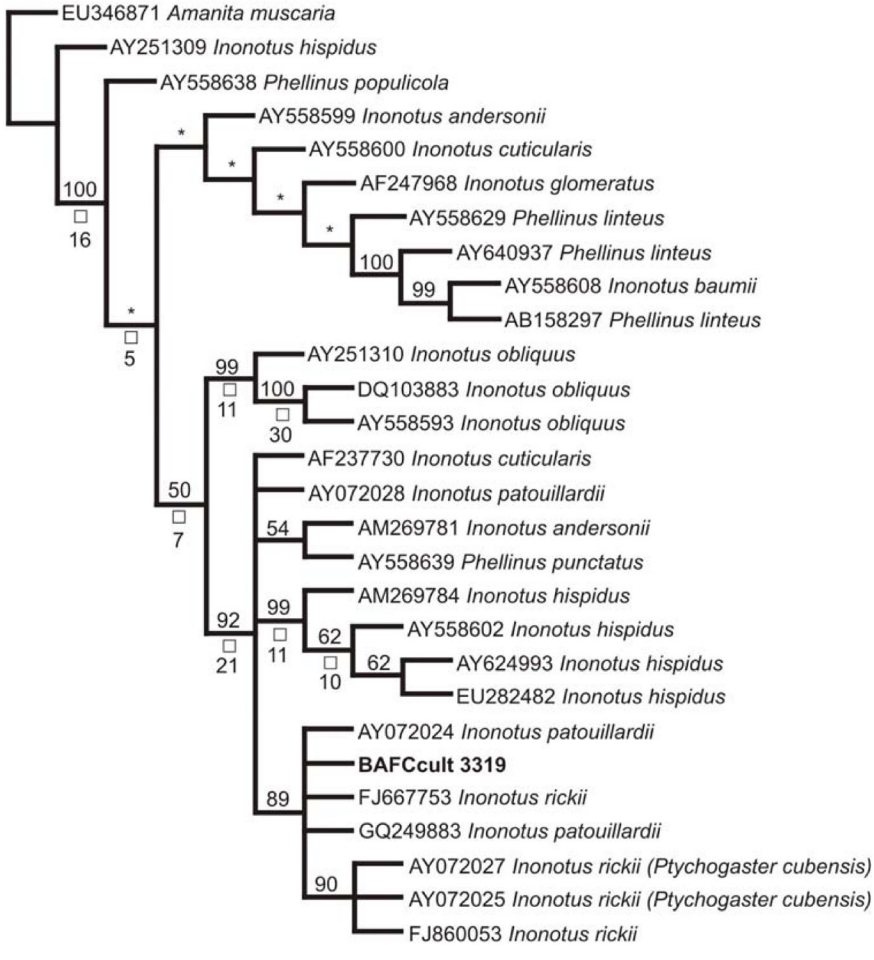

Fig. 4. The strict consensus trees obtained from *Inonotus* and *Phellinus* sequences based on ITS sequence data and maximum parsimony analysis under static homologies. Boostrap support values for 1000 replications are shown above branches. Bremer support values for default values are shown below branches. * indicates collapsed clades when Boostrap support was performed. □ indicates clades recovered by both analyses under static and dynamic homologies. In bold type is presented the sequence obtained in this work. The tree was rooted to *Amanita muscaria* (EU346871).

The strain corresponding to BAFCcult 3309 (GenBank nº FJ882040) was identified as *Peniophora laxitexta* by cultural characters. The phylogenetic analyses included sequences of several species of the genus from the Northern Hemisphere, Lithuania and Sweden. Analysis under static homologies resulted in one most parsimonious tree, tree length = 531, CI = 0.86, RI = 0.87. Analysis under dynamic homologies resulted in one retained tree, Cost = 992.

The topology of the tree sustains the species recognized for the genus. The position of the Argentinean strain is consistent with the identification of BAFCcult 3309 as a different species inside the *Peniophora* genus. To our knowledge, this is the first report of a DNA sequence of *P. laxitexta* (Fig. 5).

Phlebiopsis gigantea (Fr.) Jülich, *Persoonia* 10 (1): 137. 1978.

Descriptions: See Deschamps & Wright (1975) and Nakasone (1990).

Examined material:

ARGENTINA. Buenos Aires City, C. A. Robles, VIII-2007. On *Platanus acerifolia*. BAFC cult 3318.

Cultural studies suggested that the strain corresponding to BAFCcult 3318 (GenBank

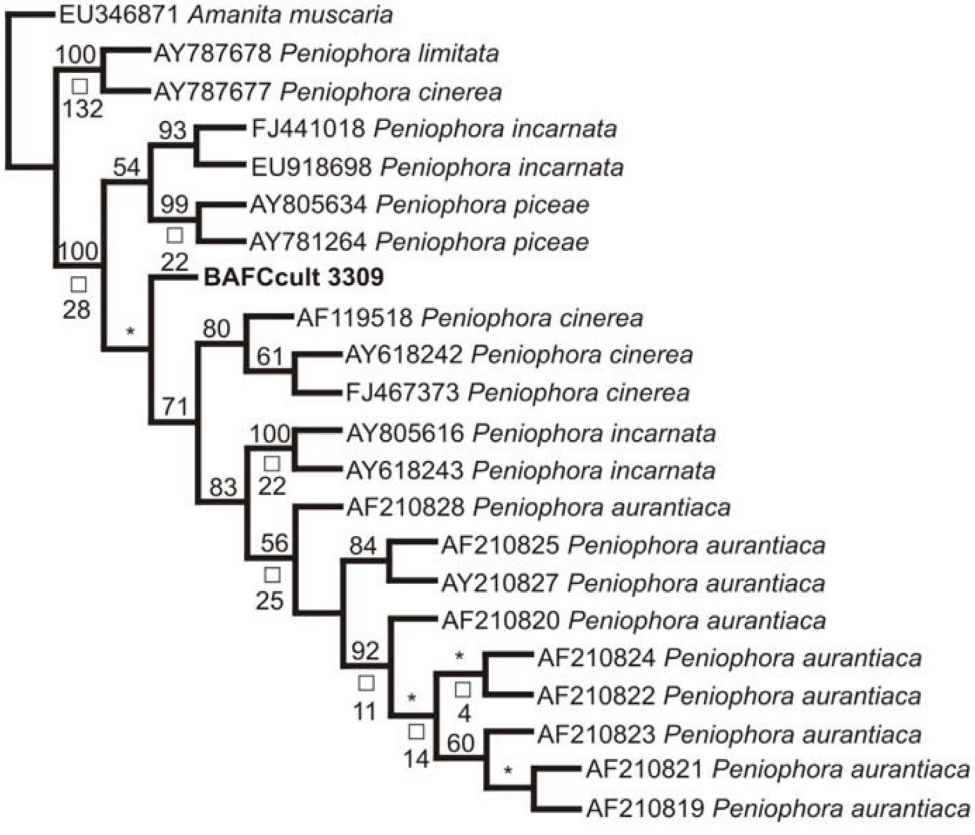

Fig. 5. The strict consensus trees obtained from *Peniophora* sequences based on ITS sequence data and maximum parsimony analysis under static homologies. Boostrap support values for 1000 replications are shown above branches. Bremer support values for default values are shown below branches. * indicates collapsed clades when Boostrap support was performed. □ indicates clades recovered by both analyses under static and dynamic homologies. In bold type is presented the sequence obtained in this work. The tree was rooted to *Amanita muscaria* (EU346871).

nº FJ850972) belongs to *Phlebiopsis gigantea*. The phylogenetic analyses included sequences of specimens from gymnosperms and from forest soil without any additional information about the host. The reports are from Canada, Finland, Lithuania, Poland, Sweden and USA. There are some reports for this species in the Southern Hemisphere (Deschamps & Wright, 1975; Vainio, 2008). Analysis under static homologies resulted in three most parsimonious trees, tree length = 315, CI = 0.75, RI = 0.59. Analysis under dynamic homologies resulted in two retained trees, Cost = 821. In both phylogenetic analyses, the Argentinean strain is associated, with a high support value, with the clade that contains most of the *P. gigantea* strains incorporated in the analyses. This result suggests that the Argentinean strain belongs to *P. gigantea* (Fig. 6). However, the strain BAFCcult 3318 remains in a separated clade from the rest of strains analyzed. *P. gigantea* is quite common in the gymnosperm-dominated forests of the northern hemisphere, so new additions of regional strains to these analyses are necessary to determinate if this topology indicates that Argentinean strains, or maybe those from the Southern Hemisphere, represents a cluster, and analyze the taxonomic implications of it.

Regarding the use of molecular data as a tool in fungal identification, it should be noted that Vialle et al (2009) evaluated several mitochondrial genes as DNA barcode but pointed out that no single mitochondrial gene gave a better taxonomic resolution than the ITS, the region already widely used in fungal taxonomy. The internal transcribed spacer (ITS) of nuclear DNA (nrDNA) has been proposed (Bellemain et al., 2010) as the official primary barcoding marker for fungi (Deliberation of 37 mycologists from 12 countries at the Smithsonian's Conservation and Research Centre, Front Royal, Virginia, May 2007).

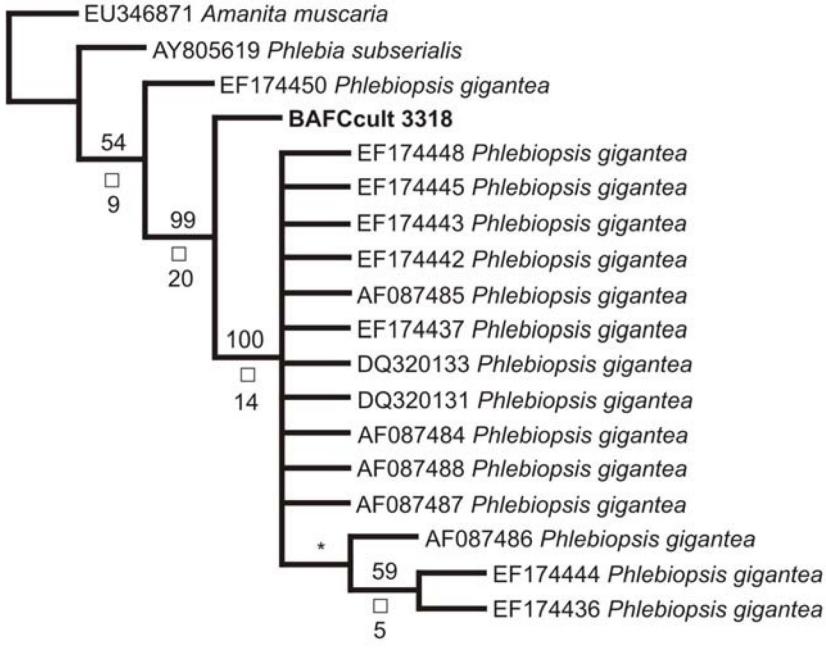

Fig. 6. The strict consensus trees obtained from *Phlebiopsis* and *Phlebia* sequences based on ITS sequence data and maximum parsimony analysis under static homologies. Boostrap support values for 1000 replications are shown above branches. Bremer support values for default values are shown below branches. * indicates collapsed clades when Boostrap support was performed. □ indicates clades recovered by both analyses under static and dynamic homologies. In bold type is presented the sequence obtained in this work. The tree was rooted to *Amanita muscaria* (EU346871).

Table 1
Bjerkandera sp. strains, GenBank accession number and origin of the sequences.
N.d.: no data available*: unpublished paper.

Species	GenBank Accession number	Geographic Origin	References
Bjerkandera adusta	AY089741	N.d.	Adair et al. (2002)
Bjerkandera adusta	FJ903311	Latvia	Arhipova et al. (2009)*
Bjerkandera adusta	FJ903353	Latvia	Arhipova et al. (2009)*
Bjerkandera adusta	FJ228211	Sweden	Bakys et al. (2009)
Bjerkandera adusta	AF455410	N.d.	Buzina et al. (2003)
Bjerkandera adusta	AF455440	N.d.	Buzina et al. (2003)
Bjerkandera adusta	AF455468	N.d.	Buzina et al. (2003)
Bjerkandera adusta	AF455521	N.d.	Buzina et al. (2003)
Bjerkandera adusta	EU918694	N.d.	Cao et al. (2008)*
Bjerkandera adusta	AY319191	Poland	Kornillowicz-Kowalska et al. (2006)
Bjerkandera adusta	FJ810147	China	Jiang et al. (2009)*
Bjerkandera adusta	FJ608590	Poland	Homolka et al. (2010)
Bjerkandera adusta	AY354210	Lithuania	Lygis et al. (2004)
Bjerkandera adusta	AY787666	Lithuania	Lygis et al. (2005)
Bjerkandera adusta	AY805605	Sweden	Menkis et al. (2004)
Bjerkandera adusta	AY805628	Sweden	Menkis et al. (2004)
Bjerkandera adusta	EF441742	Belice	Romero et al. (2007)
Bjerkandera adusta	AB096737	Japan	Sato et al. (2003)
Bjerkandera adusta	AY618221	Sweden	Vasiliauskas et al. (2004)
Bjerkandera adusta	AY781249	Sweden	Vasiliauskas et al. (2005)
Bjerkandera adusta	AJ006672	N.d.	Yao et al. (1999)
Bjerkandera adusta	DQ060097	Canada	Ze-Ze et al. (2005)*
Bjerkandera fumosa	FJ903376	Latvia	Arhipova et al. (2009)*
BAFCcult 3301	FJ850965	Argentina	Robles et al. (2011)

Table 2
Coprinopsis sp., *Coprinellus* sp. and *Psathyrella* sp. strains, GenBank accession number and origin of the sequences. N.d.: no data available.*: unpublished paper. +: published only in database. “: direct submission.

Species	GenBank Accession number	Geographic origin	References
Coprinopsis cinerea	EU168109	U.S.A.	Naumann et al. (2007)
Coprinellus curtus	AY461824	Hawaii	Keirle et al. (2004)
Coprinellus curtus	AY461834	Hawaii	Keirle et al. (2004)
Coprinellus disseminatus	FJ228213	Sweden	Bakys et al. (2009)
Coprinellus disseminatus	AY461838	Hawaii	Keirle et al. (2004)
Coprinellus disseminatus	AF345809	N.d.	Park et al. (2001)*
Coprinellus disseminatus	FN386275	Spain	Sánchez Marquez (2010)
Coprinellus flocculosus	AF345818	N.d.	Park et al. (2001)*
Coprinellus micaceus	AY461832	Hawaii	Keirle et al. (2004)
Coprinellus micaceus	AF345808	N.d.	Park et al. (2001)*
Coprinellus micaceus	EU551214	Australia	Peterson et al. (2009)
Coprinellus micaceus	EU436684	N.d.	Restrepo et al. (2008)*
Coprinellus micaceus	FN386285	Spain	Sánchez Marquez (2010)
Coprinellus radians	FJ582637	China	Jiang et al. (2008)*
Coprinellus radians	AY461815	Hawaii	Keirle et al. (2004)
Coprinellus radians	AY461818	Hawaii	Keirle et al. (2004)
Coprinellus radians	EU168103	U.S.A.	Naumann et al. (2007)
Coprinellus radians	AF345822	N.d.	Park et al. (2001)*
Coprinellus radians	AB470820	China	Tian (2009) +
Coprinellus radians	FJ462761	China	Zhang et al. (2008) * “
Coprinellus verrucispermus	AY521250	N.d.	Seok et al. (2004)*
Coprinellus xanthothrix	EU168106	U.S.A.	Naumann et al. (2007)
Coprinellus xanthothrix	AF361228	N.d.	Park et al. (2001)*
Coprinellus xanthothrix	FJ755223	N.d.	Zhao et al. (2009)*
Coprinopsis cothurnata	AY461833	Hawaii	Keirle et al. (2004)
Coprinopsis cothurnata	AF345820	N.d.	Park et al. (2001)*
Coprinopsis stercorea	AY461828	Hawaii	Keirle et al. (2004)
Coprinopsis stercorea	AY461839	Hawaii	Keirle et al. (2004)
Coprinopsis stercorea	FJ386439	United Kingdom	Navarro–Gonzalez et al. (2008)*
Coprinopsis stercorea	AF345817	N.d.	Park et al. (2001)*
Psathyrella clivensis	DQ389683	Sweden	Larsson and Örstadius (2008)
Psathyrella microrrhiza	DQ389684	Sweden	Larsson and Örstadius (2008)
Psathyrella multipedata	AM712279	Czech Republic	Vâsutová et al. (2008)
Psathyrella multipedata	AM712280	Czech Republic	Vâsutová et al. (2008)
Psathyrella spadiceogrisea	DQ389682	Sweden	Larsson and Örstadius (2008)
Psathyrella spadiceogrisea	AM712276	Czech Republic	Vâsutová et al. (2008)
BAFCcult 3218	FJ850970	Argentina	Robles et al. (2011)
BAFCcult 3311	FJ850971	Argentina	Robles et al. (2011)
BAFCcult 3312	FJ850969	Argentina	Robles et al. (2011)
BAFCcult 3313	FJ850968	Argentina	Robles et al. (2011)

Table 3
Ganoderma sp. strains, GenBank accession number and origin of the sequences.
N.d.: no data available.*: unpublished paper.

Species	GenBank Accession number	Geographic origin	References
Ganoderma adspersum	AM906054	Italy	Guglielmo et al. (2008)
Ganoderma adspersum	EF060011	Italy	Terho et al. (2007)
Ganoderma applanatum	FJ627253	N.d.	Cui (2009)*
Ganoderma applanatum	EF060006	Finland	Terho et al. (2007)
Ganoderma applanatum	AY884179	England	Wang and Yao (2005)*
Ganoderma australe	EU239390	N.d.	Glen et al. (2009)
Ganoderma australe	AY884182	England	Wang and Yao (2005)*
Ganoderma lucidum	AM269772	U.S.A.	Guglielmo et al. (2007)
Ganoderma lucidum	AM269773	Italy	Guglielmo et al. (2007)
Ganoderma lucidum	EF188278	China	Jia et al. (2006)*
Ganoderma lucidum	EF188280	China	Jia et al. (2006)*
Ganoderma lucidum	AY636058	N.d.	Singh et al. (2003)
Ganoderma lucidum	AY636059	N.d.	Singh et al. (2003)
Ganoderma lucidum	GQ249886	N.d.	Singh et al. (2009)*
Ganoderma oerstedii	DQ425011	N.d.	Su et al. (2007)
Ganoderma pfeifferi	AM269774	Italy	Guglielmo et al. (2007)
Ganoderma resinaceum	AM906060	Italy	Guglielmo et al. (2008)
Ganoderma resinaceum	AM906062	Italy	Guglielmo et al. (2008)
Ganoderma resinaceum	AM906063	Italy	Guglielmo et al. (2008)
Ganoderma resinaceum	AM906064	Italy	Guglielmo et al. (2008)
Ganoderma resinaceum	AM906065	Italy	Guglielmo et al. (2008)
Ganoderma resinaceum	AM269776	Italy	Guglielmo et al. (2008)
Ganoderma resinaceum	AM269777	Italy	Guglielmo et al. (2008)
Ganoderma resinaceum	FJ805250	N.d.	Lesage-Meessen et al. (2009)*
Ganoderma resinaceum	X78737	Netherlands	Moncalvo et al. (1995)
Ganoderma resinaceum	EF060007	Germany	Terho et al. (2007)
Ganoderma resinaceum	AY884177	England	Wang and Yao (2005)*
Ganoderma tsugae	DQ425004	N.d.	Su et al. (2007)
BAFCcult 3296	FJ850967	Argentina	Robles et al. (2011)
BAFCcult 3297	FJ850966	Argentina	Robles et al. (2011)

Table 4
Inonotus sp. and *Phellinus* sp. strains, GenBank accession number and origin of the sequences. N.d.: no data available*: unpublished paper. “: direct submission.

Species	GenBank Accession number	Geographic origin	References
Inonotus andersonii	AM269781	U.S.A	Guglielmo et al. (2007)
Inonotus andersonii	AY558599	N.d.	Jeong et al. (2005)
Inonotus baumii	AY558608	N.d.	Jeong et al. (2005)
Inonotus cuticularis	AF237730	Canada	Hamelin et al. (2002)
Inonotus cuticularis	AY558600	N.d.	Jeong et al. (2005)
Inonotus glomeratus	AF247968	Canada	Hamelin et al. (2002)
Inonotus hispidus	AY624993	Germany	Fischer et al. (2005)
Inonotus hispidus	AM269784	U.S.A	Guglielmo et al. (2007)
Inonotus hispidus	AY558602	N.d.	Jeong et al. (2005)
Inonotus hispidus	AY251309	N.d.	Cho et al. (2003)*
Inonotus hispidus	EU282482	Spain	Gonzalez et al. (2007)*
Inonotus obliquus	AY251310	N.d.	Cho et al. (2003)*
Inonotus obliquus	AY558593	N.d.	Jeong et al. (2005)
Inonotus obliquus	DQ103883	N.d.	Jiang et al. (2005)*
Inonotus patouillardii	AY072024	Argentina	Gottlieb et al. (2002)
Inonotus patouillardii	AY072028	Argentina	Gottlieb et al. (2002)
Inonotus patouillardii	GQ249883	N.d.	Singh et al. (2009)*
Inonotus rickii	FJ667753	China	Dai et al. (2010)
Inonotus rickii	AY072025	Argentina	Gottlieb et al. (2002)
Inonotus rickii	AY072027	Argentina	Gottlieb et al. (2002)
Inonotus rickii	FJ860053	China	He et al. (2009)* “
Phellinus linteus	AB158297	N.d.	Iwata (2003)[+]
Phellinus linteus	AY558629	N.d.	Jeong et al. (2005)
Phellinus linteus	AY640937	N.d.	Lee and Jung (2004)*
Phellinus punctatus	AY558639	N.d.	Jeong et al. (2005)
Phellinus populicola	AY558638	N.d.	Jeong et al. (2005)
BAFCcult 3319	GU016326	Argentina	Robles et al. (2011)

Table 5
Peniophora sp. strains, GenBank accession number and origin of the sequences.
N.d.: no data available.*: unpublished paper. “: direct submission.

Species	GenBank Accession number	Geographic origin	References
Peniophora aurantiaca	AF210819	Northern Hemisphere	Kuffer and Hallenberg (2000)
Peniophora aurantiaca	AF210820	Northern Hemisphere	Kuffer and Hallenberg (2000)
Peniophora aurantiaca	AF210821	Northern Hemisphere	Kuffer and Hallenberg (2000)
Peniophora aurantiaca	AF210822	Northern Hemisphere	Kuffer and Hallenberg (2000)
Peniophora aurantiaca	AF210823	Northern Hemisphere	Kuffer and Hallenberg (2000)
Peniophora aurantiaca	AF210824	Northern Hemisphere	Kuffer and Hallenberg (2000)
Peniophora aurantiaca	AF210825	Northern Hemisphere	Kuffer and Hallenberg (2000)
Peniophora aurantiaca	AF210827	Northern Hemisphere	Kuffer and Hallenberg (2000)
Peniophora aurantiaca	AF210828	Northern Hemisphere	Kuffer and Hallenberg (2000)
Peniophora cinerea	AF119518	N.d.	Hsiau and Harrington (2003)
Peniophora cinerea	AY787677	Lithuania	Lygis et al. (2005)
Peniophora cinerea	AY618242	Sweden	Vasiliauskas et al. (2004)
Peniophora cinerea	FJ467373	N.d.	Wu and Li (2008)* “
Peniophora incarnata	EU918698	N.d.	Cao et al. (2008)*
Peniophora incarnata	AY805616	Sweden	Menkis et al. (2004)
Peniophora incarnata	FJ441018	N.d.	Sun et al. (2008)*
Peniophora incarnata	AY618243	Sweden	Vasiliauskas et al. (2004)
Peniophora limitata	AY787678	Lithuania	Lygis et al. (2005)
Peniophora piceae	AY805634	Sweden	Menkis et al. (2004)
Peniophora piceae	AY781264	Sweden	Vasiliauskas et al. (2005)
BAFCcult 3309	FJ882040	Argentina	Robles et al. (2011)

Table 6
Phlebiopsis sp. and *Phlebia* sp. strains, GenBank accession number and origin of the sequences. *: unpublished paper.

Species	GenBank Accession	Geografic origin	References
Phlebiopsis gigantea	EF174436	Poland	Belbahri et al. (2006)*
Phlebiopsis gigantea	EF174437	Poland	Belbahri et al. (2006)*
Phlebiopsis gigantea	EF174442	Poland	Belbahri et al. (2006)*
Phlebiopsis gigantea	EF174443	Poland	Belbahri et al. (2006)*
Phlebiopsis gigantea	EF174444	Poland	Belbahri et al. (2006)*
Phlebiopsis gigantea	EF174445	Poland	Belbahri et al. (2006)*
Phlebiopsis gigantea	EF174448	Poland	Belbahri et al. (2006)*
Phlebiopsis gigantea	EF174450	Poland	Belbahri et al. (2006)*
Phlebiopsis gigantea	AF087484	Canada	Vainio and Hantula (2000)
Phlebiopsis gigantea	AF087485	USA	Vainio and Hantula (2000)
Phlebiopsis gigantea	AF087486	Finland	Vainio and Hantula (2000)
Phlebiopsis gigantea	AF087487	Canada	Vainio and Hantula (2000)
Phlebiopsis gigantea	AF087488	Canada	Vainio and Hantula (2000)
Phlebiopsis gigantea	DQ320131	Lithuania	Vasiliauskas et al. (2007)
Phlebiopsis gigantea	DQ320133	Sweden	Vasiliauskas et al. (2007)
Phlebia subserialis	AY805619	Sweden	Menkis et al. (2004)
BAFCcult 3318	FJ850972	Argentina	Robles et al. (2011)

Conclusions

Our work shows some of the scopes of ITS region as taxonomic tool. The results exhibit dissimilar situations. In the case of *Coprinellus micaceus*, phylogenetic analyses lead to the identification without basidiome or previous culture descriptions. For example in *Peniophora laxitexta*, molecular tools were not useful because there were not previous sequences of this species. In this case the study is limited by the lack of sequences in the databases. In other cases these limitations lies at regional level, as in the case of *Inonotus rickii*, *Ganoderma resinaceum* and *Phlebiopsis gigantea*. We consider that the latter is probably the commonest obstacle in the development of studies in the frame of detection of wood decay in urban trees. However, the type of analysis tested here, combined with the knowledge of the host species, can be useful for a good approach at genus level of wood-rotting fungi.

In the future, direct analyses of DNA (environmental samples) on wood would be useful only if they could be compared with sequences from correctly identified specimens, with metadata about host, information about georefence and the access to the final results in the published paper. In order to come to this point it is required to increase the size of databases with molecular data from local populations. However, it is still necessary to conduct studies involving morphological and culture aspects to complement diagnosis of wood-rot fungi to ensure the taxonomic positions of obtained sequences.

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